Methods: Individuals from Havana city, with a history of infection by D1 and D2 during the epidemics occurred in Cuba in 1977 and 1981, respectively, were studied. PBMC were isolated and ex vivo stimulated by infectious virus particles for 24 hrs. Supernatant was then collected for multiplex ELISA analysis and RNA was prepared from cells for real-time RT-PCR. The expression of the chemokines IL-8, RANTES, MIP-1 alfa, MCP-1 y CCR-1, and the cytokines IFN gamma, TGF beta, IL-10, IL-12p40, TNF alfa, IL-6, IL1 alfa and IL-1 beta, and their levels in supernatants was examined.

Results: In this study we demonstrated the strong inflammatory cell activation induced in peripheral blood mononuclear cell after 24 culture with dengue virus through the analysis of the gene expression and quantification in supernatant of IL6, IL-8, IL-1 beta, MIP-1 alfa, CCR1, MCP-1 and Rantes.

Conclusion: Given the fact that intensity of DV replication during the early times of infection could determine clinical outcomes, our results give insights about the impact of DV infection on innate immunity, the earliest defense against microbial infection, that also profoundly regulates the adaptive T-B immune responses.

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46.004

Racial Variation in the Cytokines Production During Dengue Infection

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Background: Cuban DHF/DSS outbreaks provided evidences of the reduced risk of people of Negroid race for DSS compared to Caucasoid people. These observations about the Cuban dengue outbreaks have significant epidemiological interest, as the differences in susceptibility to DHF among racial groups in Cuba coincide with the reported in African and Black Caribbean populations. In the present study the IL-10, TGF beta, IFN gamma, and TNF alfa cytokines production of Cuban donors previously infected with dengue 1 and dengue 2 during the 1977 and 1981 epidemics, and belonging to different ethnic groups, was examined.

Methods: PBMC were isolated and ex vivo stimulated by infectious virus particles for 24hrs. Supernatant was then collected for multiplex ELISA analysis and RNA was prepared from cells for real-time RT-PCR.

Results: White people showed, contrary to Blacks, a stronger and production of TNF alfa and IFN gamma, cytokines that are related with the pathogenesis of the dengue hemorrhagic fever.

Conclusion: The observed variation in the T cell response according to ethnicity could be related to the immunopathogenesis for the severest dengue clinical picture compared to Whites.

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46.005

The Effects of Multiple Passaging Regimes on West Nile Virus Genome and Infectability

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Background: The Flaviviridae family consists of several medically important pathogens such as West Nile virus (WNV), Dengue virus and Yellow Fever virus. Circulating WNV strains in recent outbreaks has been slowly evolving according to environmental pressure. We would like to decipher the effects of passaging WNV through different hosts to determine genetic regions affecting for specific host tropism.

Methods: Cell culture adapted WNV Sarafend (C-WNV) was passaged repeatedly either through suckling mice brain (S-WNV) or a serially through suckling mice followed by mosquito cell culture (SM-WNV). The resultant virus after ten rounds was sequenced and tested for pathogenicity effects on suckling mice, adult mice, and primary cell culture.

Results: This study found WN virus becoming more virulent after passaging in the suckling mouse-mosquito cell culture line (SM-WNV) compared to the unpassaged virus. The virus acquired a smaller plaque size, killed suckling mice faster than suckling mouse passaged virus (S-WNV), and could infect primary neuronal cells at a higher affinity and produced higher virus titers. Partial sequencing the virus genome before and after the passaging experiments pointed out regions of point mutations that occurred during the adaptation process that could have led to the phenotypic changes. We postulate that the serial passaging through two different cell species mimicked natural virus life cycles and in effect increased virus robustness. The virus was thus more able to adapt to the host mice conditions.

Conclusion: This result could translate into a tool for WNV drug targeting as we now recognise genetic areas where mutations are more likely to occur for the efficient adaptation of the virus to a new host. Molecules targeting these sites would in effect hamper the virus from causing serious disease or adapting to new species.

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46.006

West Nile Virus Capsid and viral RNA interaction

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Background and Aim: The process of assembly of the West Nile (WN) virus presents itself as an attractive anti-viral tar-